A further Account of the Spermatogenesis of Lice.

By

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With 1 Text-figure.

In 1919 the late Professor Doncaster and the present author investigated the gametogenesis of the lice Pediculus corporis and Pediculus capitis. An account of this work was published during the following year (3). The cytology of the spermatogenesis was found to be so peculiar and in some respects unique, that it was decided to extend the work to other lice. At the time of his death Professor Doncaster was working on the gametogenesis of the horse-louse and the dog-louse. The material collected by him together with his notes was handed over to me by Professor J. Stanley Gardiner.

A perusal of the notes showed that Doncaster had only cursorily examined his material and had not arrived at any new conclusions. For this reason it was not considered advisable to publish any further account based on the new material. However, at the end of 1919 an account of the spermatogenesis of Pediculus vestimenti was published by Miss Foot (4) which differed so considerably from that published by Doncaster and myself that a thorough examination of Doncaster's new material seemed called for, and the present note is an account of that work.

The material examined consisted of testes of the dog-louse (Lignognathus piliferus) and of the horse-louse (Haematopinus asini). Some fixed material of the last-named species, obtained from a donkey, was kindly sent to me recently by Mrs. Bisbee of Liverpool University, who was assisting Professor Doncaster in this work. I also obtained

some specimens of Haematopinus consobrinus from a monkey that died in the Zoological Gardens.

As with Pediculus, the material was found to be extremely refractory with regard to fixation. The best fixation generally was obtained with Flemming and Flemming without acetic. For the various stages of the mitosome Mann's corrosive osmic fixative gave excellent results. Kopsch's method of prolonged fixation in osmic was found to be useless—the unstained sections showing nothing impregnated. For staining—as with Pediculus—the only stain of any considerable use was Heidenhain's iron haematoxylin. Altmann's methyl green-fuchsin was used for some of the material but did not give results of much value.

The most important points in the spermatogenesis of Pediculus as described by Doncaster and myself are as follows: (1) While the somatic chromosome number is twelve the spermatogonial figures show only six. This apparently haploid number of chromosomes in the spermatogonia we ascribed to premature pairing. (2) There is only one spermatocyte division, which is extremely unequal, leading to the separation of a minute 'polar body-like' cell which degenerates. (3) The centrosome of the spermatid is double and from each half an axial filament grows out so that the developing spermatozoa have two conspicuous axial filaments.

The account given by Miss Foot differs from our description in the following points: (1) The somatic number of chromosomes is stated to be ten, and in the few spermatogonial groups found it is also stated to be ten. (2) There is always an unequal bivalent in the first spermatocytes. (3) Although the division of the second spermatocytes was not observed, it is assumed that this division is similar to that in corresponding mitoses in 'other species of Hemiptera'.

With regard to the chromosome number, Miss Foot states that the chromosomes are so small and so frequently constricted that the estimated number can always be questioned. The chromosomes certainly are very small, but in equatorial plates of follicle cells and of cells that we called spermatogonia we

experienced no difficulty in making an accurate count of the chromosomes, and, as we stated, the frequency with which we were able to make these counts can leave no doubt as to the actual number of chromosomes. No evidence was found of an unequal pair. The sizes of the chromosomes varied somewhat and this, combined with their smallness, would have made it possible to postulate dimorphism in the chromosomes only if the difference in size were very marked.

The third difference between the two accounts, namely that referring to the unequal spermatocyte division, is the most important. Miss Foot maintains that the first spermatocyte chromosomes of Pediculus are very similar to those of Euschistus, and states that as the first spermatocyte chromosomes 'have the same morphological characteristics as the corresponding stages in other species of Hemiptera, it is logical to assume that the second spermatocyte chromosomes would be equally typical'. Unfortunately the paper is not illustrated by the usual excellent photographs which characterize so many of Miss Foot's works, and it is difficult to compare her Pediculus figures with her series of photographs of spermatocyte divisions of Euschistus. However, by comparing our preparations of Pediculus with Miss Foot's Euschistus photographs I have to confess that I cannot see any resemblance at all.

Further, Miss Foot's observations were made on smear preparations. In such preparations I think one may say that it is highly probable that spermatocytes dividing in such an unequal manner as we described would be so distorted, if not completely collapsed, as to be unrecognizable. The small polar body-like cell which is given off from the spermatocyte is, at the moment of its formation, a long finger-like process. In a smear preparation it is most probable that this attenuated process would be either torn away from the remainder of the dividing cell or else the whole cell on being freed from the surrounding cells would round itself off and appear as a cell in equal mitosis.

Sections of the testis of Pediculus show an orderly sequence of stages from the spermatogonia at the free end to the fully

formed spermatozoa at the thicker end nearer the vas deferens. The position of a cell is thus to some extent an indication of the stage of its maturity. In smear preparations this orderly arrangement is obliterated, and hence it appears to me it would be an easy matter to overlook the unequal spermatocyte divisions in such preparations, even if they were demonstrable, unless their presence were suspected.

Another aid in working out the spermatogenesis is the development of the cytoplasmic inclusions, especially the mitosome, *pari passu* with the maturation stages. It is significant that Miss Foot does not mention or figure the mitosome which is so conspicuous in the later stages of spermateleosis.

From the examination of the new louse material it is clear that the course of spermatogenesis in the three species observed is, in its main features, the same as that in *Pediculus corporis* and *Pediculus capitis* as described by Doncaster and myself, and does not agree with the process as described by Miss Foot. This fact is in itself strong indirect evidence in support of our original description.

The testes of the three species examined were all similar to those of Pediculus, those of *H. consobrinus* being somewhat more pear-shaped and less ovoid. The arrangement of the follicles of cells was the same in all cases, the spermatogonia being at the free end and the mature spermatozoa being found at the broader end at the entrance to the vas deferens. A conspicuous mitosome is formed in the spermatid by the coalescence of the mitochondria as in Pediculus. In the horse-louse all stages of the single spermatocyte divisions were found, and these were, in all essentials, closely similar to those in Pediculus. The metaphase spindle is always eccentric, the main part of the cell being occupied by the mitochondrial mass. During anaphase the spindle elongates very considerably, and one pole, with its centrosome, is carried outwards from the main body of the cell on a long finger-like projection extending outwards to a distance of the diameter of the cell. This process, with its contained chromosomes, breaks away from the body of the cell, forming a minute ' polar body-like ' cell.

In the dog-louse and in H. consobrinus I have been unable to find the spermatocyte divisions, in the latter case perhaps because my material was scarce; but in the former case all mitoses were extremely rare. As, however, all the other stages of spermatogenesis in the two forms corresponded closely to those of the horse-louse and of Pediculus, it is logical to infer that the missing spermatocyte divisions will be of the same type.

In the horse-louse the number of chromosomes in spermatogonial metaphase plates is nine. Spermatocyte prophases indicate a similar number, but not so clearly. In anaphase the chromosomes are too clumped to count with certainty. In H. consobrinus the spermatocyte prophases show seven chromosomes. In the dog-louse no reliable count was obtained.

After the telophase of the spermatocyte division of the horse-louse the centrosome appears to double as in Pediculus, and from this double centrosome the double axial filament of the tail of the spermatozoa commences to grow out. At this stage the chromatin of the nucleus appears in a clumping which is very irregular but in which a conspicuous nucleolus is always present (Text-fig. I). As the spermatid elongates the nucleolus becomes attached to the nuclear membrane so that it projects partly in and partly out of the nucleus (Text-fig. J). It usually appears near the apex of the spermatid but its position is not definite. As spermateleosis proceeds all the staining material in the nucleus disappears, and with it the nucleolus becomes gradually smaller and also disappears (Text-fig. L). This process does not take place in the dog-louse nor in H. consobrinus. In Pediculus we described a deeply-staining body which lies within the nucleus at the posterior end of the heads of fairly late spermatids. This does not occur in any of the new lice examined, and probably corresponds to the late appearance of the nucleolus described here in the horse-louse.

In Pediculus we described a body which we provisionally called the 'acroblast'. In referring to acrosome formation generally, Gatenby and Woodger (**8**) state 'according to the account given for Smerinthus by Gatenby (**6**) and for Pediculus

by Doncaster and Cannon (**3**) all the Golgi apparatus is taken up in the formation of the acrosome '. This statement might pass

TEXT-FIG. 1.

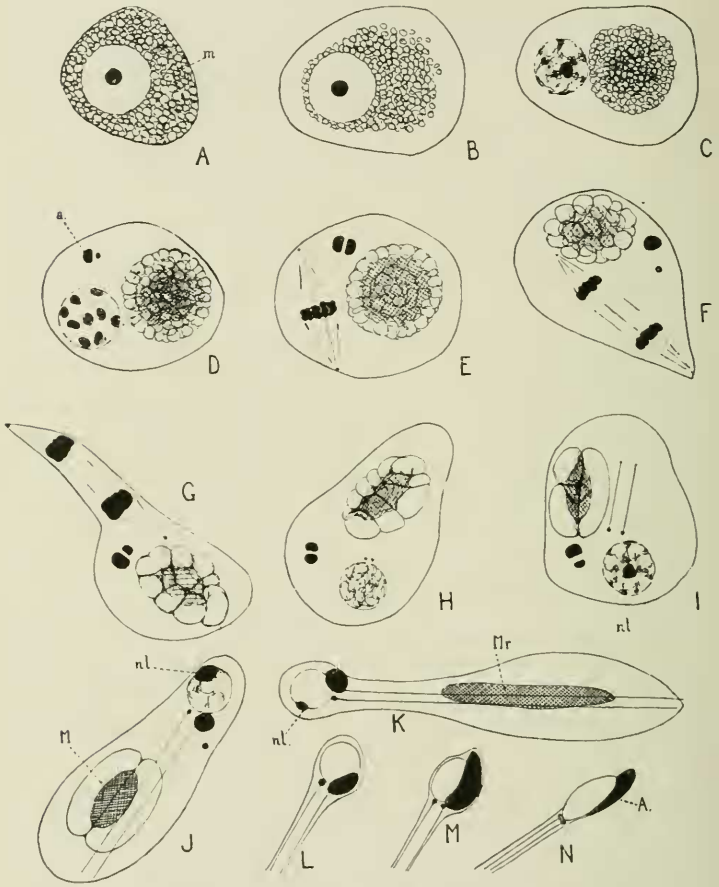

Diagram illustrating the spermatogenesis of lice. *a.*, acroblast. *A.*, acrosome. *m.*, mitochondria. *M.*, mitosome. *Mr.*, mitosome remnant. *nl.*, nucleolus.

without comment were it not that it is grossly inaccurate. We stated ' we have been unable to determine with certainty either the origin or the nature of the " acroblast ", but on the analogy of the bodies in Lepidoptera, which Gatenby calls by

the same name, we suspect that it belongs to the Golgi apparatus. Attempts to prove this by Kopsch's method . . . have, however, failed to confirm this belief. . . .' We took this view that the body with which we were dealing was probably the 'acroblast' on account of its behaviour in moving ultimately towards the tip of the developing spermatozoon, and we associated it with the Golgi apparatus because we were convinced that the 'acroblasts' of Gatenby were really the Golgi bodies of earlier writers—a conclusion at which Gatenby ultimately arrived—not in the paper on Smerinthus quoted by Gatenby and Woodger, in which no mention is made of the Golgi apparatus, but in a paper published two years later (7).

In the three species of lice examined there is, in each case, a conspicuous body which corresponds to the 'acroblast' of Pediculus. In the horse-louse its history could be made out most clearly. It first appears just prior to the prophase of the spermatocyte division (Text-fig. D). In Pediculus we stated that the 'acroblast' may sometimes be double at the time of its first appearance, but is always a single spherical body later. In the horse-louse it is usually, but not always, double, and remains so until the spermatid has formed and is elongated. Its appearance is very striking and its shape somewhat difficult to describe. Its two parts are sometimes equal (Text-fig. H), but more usually one is smaller than the other (Text-fig. D and J). The shape of each half may best be described as 'bun-shaped'. They are placed with their flat sides together but are never touching. They are always separated somehow by a transparent acromatic layer. Sometimes the two halves are seen to be comparatively far apart (Text-fig. F and J). If the difference in size between the two halves is great the smaller half is almost spherical. In the elongated spermatid just before the nucleolus finally disappears the acroblast is a single body (Text-fig. K) placed against the nucleus close to the double centrosome, as in Pediculus. It thus looks highly probable that during spermateleosis the double acroblast loses one of its halves, which passes away from the nucleus and

disappears. The stages in which the two halves are far apart (Text-fig. F and J) very probably illustrate this process taking place.

In a recent paper on the sperm of Hemiptera Bowen (**1**) enumerates fifteen cases by other authors besides his own description in which the acroblast of the spermatid in giving rise to the acrosome, which forms the tip of the spermatid, gives off a body termed by him 'the Golgi remnant', which is lost in the protoplasm of the tail region of the spermatid. Probably the case of the horse-louse must be added to this list.

In the dog-louse and in H. consobrinus the acroblast is single as in Pediculus.

Whether or not this body which we tentatively called the acroblast is really the homologue of the Golgi apparatus in other cells cannot be said with certainty until a more precise definition of the Golgi bodies is found. In the dog-louse in Mann-Kopsch preparations there are indications of the acroblast arising from two or three scattered granules which may be the true Golgi bodies. However, these are not impregnated by prolonged fixation with osmic acid. A character that is as specific of Golgi bodies as is their staining reactions is that they always show a definite relation to the centrosome during mitosis. It is significant that in all the lice examined the acroblast is peculiar in that it exists as such during the spermatocyte division, and also it does not show any definite spacial relations to the centrosomes of the dividing cell.

With regard to the mitochondria, preparations of dog-louse material fixed in Mann-Kopsch completely confirmed the account that we gave of the development of the mitosome in Pediculus. There was a slight difference in that the earliest spermatogonia in the dog-louse showed the cytoplasm completely filled with vacuolated mitochondria, whereas in Pediculus Professor Doncaster was of the opinion that some of the earliest spermatogonia showed granular mitochondria. However, the gradual fusion of these mitochondrial vacuoles to form a mitosome consisting of a central chromophilic mass surrounded by two large vacuoles took place exactly as in Pediculus and will not

be described further here. The process is figured in Text-figs. A–J.

We pointed out that the appearance of the mitochondrial mass that we described was ascribed by Gatenby to faulty fixation. This author favours the view rather, that the apparently vacuolated mitosome is the result of faulty fixation on the 'spireme' type of mitosome that he describes in Lepidoptera (**5**). In a recent paper of Bowen's (**2**). 'On the structure of the "Nebenkern" in the insect spermatid', there is a review of the work on this subject, and from this it is clear that the description given by practically all other workers, besides the very exact account given by Bowen himself, agrees closely with the development of the mitosome as we described it in Pediculus.

Apart from the fact that Gatenby's results have not been confirmed by any other worker, there are several points in his original description of the origin of the 'spireme' mitosome which make one cautious in accepting his views. He states 'that the spireme is formed from the chromophile rim (outer layer) of the mitochondrial body, while the substance, in which the spireme lies, is the coalesced inner substance (chromophobe part) of the mitochondrial layer'. He gives three diagrammatic figures illustrating the process by which this transformation is brought about and these figures are certainly very misleading. In the first one are drawn optical sections of spherical mitochondrial granules, in the other two these granules are shown elongating and fusing together and apparently thus forming, first of all loops, and finally a spireme. Now if two bodies with a chromophilic outer layer and a chromophobic inner substance coalesce, whether they are elongated or not, they merely form a larger body of chromophobic inner substance with a larger chromophilic outer layer. They do not form a thread of chromophilic substance in a mass of chromophobic substance, at least not by the mere act of coalescence, as Gatenby's figure indicates. What these figures really show is the lengthening of the optical sections of spheres, which are of course circles, to form loops, and their joining together to

form a continuous thread. This process may be possible : but what Gatenby has overlooked is the fact that the circles in his first figure, and presumably the drawn-out loops in his second figure, do not actually represent loops of thread at all but surfaces.

I do not wish to maintain that it is impossible to obtain from a system of mitochondrial bodies, such as Gatenby describes, a mitosomal spireme. If it is imagined that the chromophilic substance disappears from the interfaces between the adhering bodies and remains in the interspaces then some sort of a network of chromophilic substance would be obtained which might be described as a spireme, but this would not be a continuous single thread but a much-branching system of threads.

In viewing the mitosome, at any level of focus, one sees a coiled thread-like mitosome just as Gatenby figures, but on focusing up and down one is able to see that without any doubt it is actually a plate work formed by a system of vacuoles.

Summary.

The main results of the examination of cytological preparations of the testes of the horse-louse (Haematopinus asini), of the dog-louse (Lignognathus piliferus), and of Haematopinus consobrinus may be summarized briefly.

1. In all main points the spermatogenesis of these three species of louse agrees with that described for Pediculus corporis and Pediculus capitis by the late Professor Doncaster and the present author. Miss Foot's account of the spermatogenesis of Pediculus vestimenti is criticized.

2. In the elongating spermatid of the horse-louse, the nucleolus appears for a short period as a chromatic mass adhering to the nuclear membrane, projecting partly in and partly out of the nucleus.

3. The acroblast of the horse-louse is usually a double body consisting of two 'bun-shaped' halves which are sometimes of equal size, and which are separated with flat sides together

by a transparent achromatic layer. The 'acroblast' exists as such during the single spermatocyte division, and finally forms the 'acrosome' of the spermatid.

4. The description of the mitosome given for Pediculus corporis is substantiated by an examination of that of the dog-louse. Gatenby's description of a 'spireme mitosome' is criticized.

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May 6, 1922.

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